216547US0CONT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF: :

Keiichi YOKOYAMA et al : ATTN: Application Branch

SERIAL NO: New Application :

FILED: Herewith :

FOR: PROCESS FOR PRODUCING MICROBIAL TRANSGLUTAMINASE

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Prior to examination on the merits, please amend the above-identified application as follows:

IN THE SPECIFICATION

Please amend the specification as shown in the attached marked-up copy to read as follows:

Page 1, after the Title, insert

--This application is a Continuation of U.S. Application Serial No. 09/448,310 filed November 24, 1999, now allowed, which is a Continuation of U.S. Application Serial No. 09/109,063, filed on July 2, 1998, now US Patent No. 6,013,498.--

Page 8, line 19 through page 9, line 8, replace the text in its entirety with the following:

The DNA of the present invention encodes the above-mentioned proteins. Among these, the preferred is a DNA wherein a base sequence encoding for Arg at the fourth position from the N-terminal amino acid is CGT or CGC, and a base sequence encoding for Val at the fifth position from the N-terminal amino acid is GTT or GTA. Furthermore, the preferred is a DNA wherein a base sequence encoding for the N-terminal amino acid to fifth amino acid, Seq-Asp-Asp-Arg-Val (SEQ ID NO: 60), has the following sequence

Ser: TCT or TCC

Asp: GAC or GAT

Asp: GAC or GAT

Arg: CGT or CGC

Val: GTT or GTA

In this case, the preferred is a DNA wherein a base sequence encoding for amino acid sequence of from the N-terminal amino acid to fifth amino acid, Ser-Asp-Arg-Val (SEQ ID NO: 60), has the sequence TCT-GAC-GAT-CGT-GTT (SEQ ID NO: 61).

Page 15, line 22 through page 16, line 2, replace the text in its entirety with the following:

In fact, a sequence of Met-Ser-Asp-Asp-Arg- . . . . . (SEQ ID NO: 62) was designed by deleting N-terminal aspartic acid residue from transglutaminase derived from microorganism (MTG), and this was produced in E. coli. As a result, methionine residue was efficiently removed and thereby there was obtained a protein having a sequence of Ser-Asp-Asp-Arg- . . . . (SEQ ID NO:1, residues 2-5). It was confirmed that the specific activity of the thus-obtained protein is not different from that of natural MTG.

Please delete the original Sequence Listing at pages 27-63 without prejudice.

Page 67, after the last line, beginning on a new page, please insert the attached substitute Sequence Listing.

IN THE CLAIMS

Please cancel Claims 1-25 and add the following claims.

--26. (New) A DNA which encodes a protein having transglutaminase activity, wherein the amino acid sequence of the protein comprises the serine residue at the second position to proline residue at the 331st position of the amino acid sequence of SEQ ID NO: 1, wherein the N-terminal amino acid of the protein is the serine residue at the second position of SEQ ID NO: 1.

27. (New) The DNA of Claim 26, wherein the amino acid sequence of the protein consists of the serine residue at the second position to proline residue at the 331st position of the amino acid sequence of SEQ ID NO: 1.

28. (New) The DNA of Claim 26, wherein the base sequence encoding for Arg at the forth position from the N-terminal amino acid is CGT or CGC, and the base sequence encoding for Val at the fifth position from the N-terminal amino acid is GTT or GTA.

29. (New) The DNA of Claim 28, wherein the base sequence encoding for from the N-terminal amino acid to the fifth amino acid, Ser-Asp-Asp-Arg-Val, has the following sequence:

Ser: TCT or TCC,

Asp: GAC or GAT,

Asp: GAC or GAT,

Arg: CGT or CGC, and

Val: GTT or GTA.

30. (New) The DNA of Claim 29, wherein the base sequence encoding for an amino acid sequence of from the N-terminal amino acid to the fifth amino acid, Ser-Asp-Asp-Arg-Val, has the sequence TCT-GAC-GAT-CGT-GTT.

31. (New) The DNA of Claim 29, wherein the base sequence encoding for an amino acid sequence of from the sixth amino acid to the ninth amino acid from the N-terminal amino acid, Thr-Pro-Pro-Ala, has the following sequence:

Thr: ACT or ACC,

Pro: CCA or CCG,

Pro: CCA or CCG, and

Ala: GCT or GCA.

32. (New) The DNA of Claim 30, wherein the base sequence encoding for an amino acid sequence of from the sixth amino acid to the ninth amino acid from the N-terminal amino acid, Thr-Pro-Pro-Ala, has the following sequence:

Thr: ACT or ACC,

Pro: CCA or CCG,

Pro: CCA or CCG, and

Ala: GCT or GCA.

33. (New) A DNA comprising a nucleotide sequence ranging from the thymine base at the fourth position to the guanine base at the 993rd position of the nucleotide sequence of SEQ ID NO: 2.

34. (New) A DNA consisting of a nucleotide sequence ranging from the thymine base at the fourth position to the guanine base at the 993rd position of the nucleotide sequence of SEQ ID NO: 2.

35. (New) A recombinant DNA comprising the DNA of Claim 26.

36. (New) A recombinant DNA having a DNA of Claim 28.

37. (New) A recombinant DNA having a DNA of Claim 29.

38. (New) The recombinant DNA of Claim 35, further comprising a promoter selected from the group consisting of trp, tac, lac, trc, λPL and T7.

39. (New) The recombinant DNA of Claim 36, further comprising a promoter selected from the group consisting of trp, tac, lac, trc, λPL and T7.

40. (New) The recombinant DNA of Claim 37, further comprising a promoter selected from the group consisting of trp, tac, lac, trc, λPL and T7.

41. (New) A procaryotic microorganism transformed with the recombinant DNA of Claim 35.

42. (New) The transformed procaryotic microorganism of Claim 41, which is *Escherichia coli*.

43. (New) The transformed *Escherichia coli* of Claim 42, which is transformed with a multi-copy vector.

44. (New) The transformed *Escherichia coli* of Claim 42, wherein the *Escherichia coli* is the JM109 strain.

45. (New) A process for producing a protein having a transglutaminase activity, comprising culturing the transformed procaryotic microorganism of Claim 41 in a medium to produce the protein having the transglutaminase activity, and recovering the protein.

46. (New) A process for producing a protein having a transglutaminase activity, comprising culturing the transformed *Escherichia coli* of Claim 42 in a medium to produce the protein having the transglutaminase activity, and recovering the protein.

47. (New) A process for producing a protein having a transglutaminase activity, comprising culturing the transformed *Escherichia coli* of Claim 43 in a medium to produce the protein having the transglutaminase activity, and recovering the protein.

48. (New) A process for producing a protein having a transglutaminase activity, comprising culturing the transformed *Escherichia coli* of Claim 44 in a medium to produce the protein having the transglutaminase activity, and recovering the protein.

49. (New) A DNA which codes for a protein having transglutaminase activity and comprising an amino acid sequence represented by SEQ ID No. 1, wherein the base sequence coding for Arg at the fifth position from the N-terminal amino acid is CGT or CGC, and the base sequence coding for Val at the sixth position from the N-terminal amino acid is GTT or GTA.

50. (New) The DNA of claim 49, wherein the base sequence coding for an amino acid sequence of from the second amino acid to the sixth amino acid from the N-terminal amino acid, Ser-Asp-Asp-Arg-Val (SEQ ID NO:1, residues 2-6), has the following sequence:

Ser: TCT or TCC

Asp: GAC or GAT

Asp: GAC or GAT

Arg: CGT or GCG

Val: GTT or GTA.

51. (New) The DNA of claim 51, wherein the base sequence coding for an amino acid sequence of from the second amino acid to the sixth amino acid from the N-terminal amino acid, Ser-Asp-Asp-Arg-Val (SEQ ID NO:1, residues 2-6), has the sequence TCT-GAC-GAT-CGT-GTT (SEQ ID NO:2, bases 4-18).

52. (New) The DNA of claim 50, wherein the base sequence coding for an amino acid sequence of from the seventh amino acid to the tenth amino acid from the N-terminal amino acid, Thr-Pro-Pro-Ala (SEQ ID NO:1, residues 4-7), has the following sequence:

Thr: ACT or ACC

Pro: CCA or CCG

Pro: CCA or CCG

Ala: GCT or GCA.

53. (New) The DNA of claim 51, wherein the base sequence coding for an amino acid sequence of from the seventh amino acid to the tenth amino acid from the N-terminal amino acid, Thr-Pro-Pro-Ala (SEQ ID NO:1, residues 4-7), has the following sequence:

Thr: ACT or ACC

Pro: CCA or CCG

Pro: CCA or CCG

Ala: GCT or GCA.--

SUPPORT FOR THE AMENDMENTS

Newly added Claims 26-48 are supported by the specification at pages 5-63 and by Claims 1-25 as originally filed. Newly added Claims 49-53 are supported by original Claims 5-8, except that the N-terminal amino acid is Asp. This is supported by SEQ ID NO: 1 of the present application where Asp is the first amino acid. No new matter is believed to have been added to this application by these amendments.

REMARKS

Claims 26-53 are active in this application.

This application is a continuation of U.S. Application Serial No. 09/448,310, filed November 24, 1999, now allowed, which is a Continuation of U.S. Application Serial No. 09/109,063, filed on July 2, 1998, now U.S. Patent No. 6,013,498.

Applicants note that the claims of the present application are the same as parent case U.S. Application, Serial No. 09/448,310. The present application has been filed, in part, to obtain consideration of Devlin et al (Gene, vol. 65, 1988, pages 13-22) cited in the in the Information Disclosure Statement submitted herewith. This reference was submitted in the parent case.

The specification has been amended to insert Sequence identifiers (SEQ ID NO:) and to insert the attached Sequence Listing.

The paper copy of the Sequence Listing filed in this application is identical to the last-filed computer readable Sequence Listing in application 09/109,063 filed July 2, 1998. In accordance with 37 CFR § 1.821 (e), Applicants request that the Patent Office use the last-filed computer readable form filed in that application as the computer readable form for the instant application. It is understood that the Patent and Trademark Office will make the necessary change in application number and filing date for the instant application.

Applicants submit that the present application is ready for examination on the merits.

Early notice to this effect is earnestly solicited.

Respectfully submitted,

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Marked-Up Copy
Serial No: New Application
Amendment Filed on:
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Claims 26-53 are added.